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Monomer Networking Activates Recombinases

Recombinases catalyze the DNA strand-exchange reactions of homologous recombination. The regularity of the filaments they form on DNA, though beautiful, does not explain how they work. Functionally important internal interactions now include multiple intermonomer contacts for activation (Galkin et al., 2006).

Recombinases catalyze ATP-dependent exchange of base-paired partners between homologous DNA molecules in reactions that involve binding single-stranded DNA, identifying a homologous duplex, and switching one strand of the duplex for the bound single strand. Filaments assembled by recombinases on DNA are among the most intensely studied molecular machines, and yet their functional mechanism remains mysterious. The sheer beauty of recombinase-DNA nucleoprotein filaments, a right-handed helix of proteins (Story et al., 1992) bound to the right-handed helix of DNA, was reason enough to assume that symmetry and regularity would be essential elements of recombination function. An appreciation of filament variation and flexibility came later, after careful analyses of different structures reconstructed from EM images and comparison of multiple higher resolution crystal structures as they became available (Wyman and Kanaar, 2004; Yu et al., 2004). Notably, the pitch of the protein filament is quite variable and correlates in general with catalytic activity: filaments with longer pitch being active and those with shorter pitch being inactive (Yu et al., 2001; VanLoock et al., 2003; Wyman and Kanaar, 2004). Work described in this issue of *Structure* (Galkin et al., 2006) identifies multiple protein-protein contacts that influence filament architecture and demonstrates their importance for the catalytic activity of Rad51/RadA in ATP hydrolysis and possibly also in strand exchange.

Specifically, Galkin et al. (2006) use a combination of structural methods to address the function of the N-terminal domain of eukaryotic Rad51 and archaeal RadA recombinases. Recombinases, like many other proteins, are modular, consisting of a common structurally similar core that binds and hydrolyzes ATP, and another domain—N-terminal for archaeal RadA and eukaryotic Rad51 or C-terminal for bacterial RecA (the additional unique N-terminal extension of yeast Rad51 is not considered here). These extra domains are not structurally similar, and their function is so far enigmatic. Numerous studies have shown the relative orientation of the ATPase core and the N- or C-terminal domain to be quite variable, facilitated by the flexible amino acid stretch connecting the two. Two different recombinase versions with defective N-terminal domains, RadA missing its 62 amino acid N terminus (RadA-Δ62) and yeast Rad51 with a specific N-terminal domain point mutation (Rad51-G103E), were analyzed in order to understand the role of the N-terminal domain in catalytic function. In both cases, these proteins cannot catalyze strand exchange. The obvious possibility that tampering with the N terminus resulted in a misfolded ATPase domain and an inability to assemble into filaments was first put to rest for both protein variants by a combination of X-ray crystallography and EM image reconstruction. The specific features of the filaments they form revealed new functionally significant elements. RadA-Δ62 formed double, two-start filaments that are active in ATP hydrolysis, whereas Rad51-G103E filaments appeared normal, but apparently catalyze only one round of ATP hydrolysis.

The structural feature of these filaments that correlated with ATPase activity was multiple contacts between monomers. ATP hydrolysis by recombinases occurs at the interface between two monomers in the DNA bound protein filament (VanLoock et al., 2003; Conway et al., 2004; Wu et al., 2004). Additional contact between adjacent monomers via the N-terminal domain of one and the ATPase core of the next was evident in crystal structures of active Rad51 and RadA filaments (Conway et al., 2004; Wu et al., 2004), but not proven to have a specific function. RadA-Δ62 filaments, clearly missing the N-terminal domain interface, appear to compensate through extra contacts among monomers in the two-start, double, helix. A monomer contacts not only adjacent partners along one helix, but also monomers above and below it in the other helix. In the Rad51-G103E filaments, adjacent monomers bind ATP in an apparently catalytically active orientation; however, the N terminus was not resolved. From these experiments, the model that emerges is that effective ATP hydrolysis requires additional contact between monomers provided by the N-terminal domain or substituted by other contacts in the two-start recombinase helix.

Two functionally important connections between recombinase monomers in a filament have now been defined. An ATP hydrolysis cycle involves changes at the catalytic site interface. As suggested by Egelman and colleagues (Galkin et al., 2006), the additional interface would stabilize these transitions, facilitating multiple rounds of ATP hydrolysis. Two interfaces and their flexible connection allow for disruption of the ATPase interface while preventing monomer dissociation from the filament. The flexible arrangement of recombinase domains in filaments hydrolyzing ATP may partially explain their irregular appearance in SFM images (Ristic et al., 2005).

ATPase interfaces with different orientations (Conway et al., 2004) and additional connections involving the N-terminal domain of recombinases in filaments are also found in hexameric helicases such as T7 Gene 4 (Singleton et al., 2000). Here, helicase activity is accompanied by alternating rearrangement of monomers in the protein ring coupled to ATPase hydrolysis at their interfaces. In the linear recombinase filament, similar reorientation of monomers coupled to ATP hydrolysis would propagate architectural changes along the filament.

Bound DNA would be alternately ordered or not for single strands and stretched and released for double strands as the pitch and helical order of the protein filament changed.

DNA can also be described as a flexible scaffold to hold recombinase molecules at high concentration in a linear array. This suggests a mechanism for recombinase filaments acting as springs tensing and relaxing bound DNA. The flexible and unstructured single-stranded DNA in a filament would be alternately stretched/ordered and relaxed/less-ordered depending on the ATP hydrolysis status and therefore regular arrangement of the bound recombinases. The stiffer, structured double-stranded DNA, resulting from recombinase-mediated homologous pairing, is stretched and unwound within the filament. Disrupting the protein helix when ATP is hydrolyzed allows the DNA to relax, preventing easy reassembly, favoring monomer dissociation, and driving the reaction to favor the recombined DNA product. This, at the moment highly speculative, scenario is also only part of the recombination story. High-resolution information on DNA structures within filaments and description of recombinase dynamics within filaments will soon provide better ideas of how recombination works. Thus, even after more than two decades of intense study, recombinase nucleoprotein filaments are still among the most intriguing molecular machines around.

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Human CD23: Is It a Lectin in Disguise?

The crystal structure of a low-affinity human IgE receptor, CD23, is reported by Wurzburg et al. (2006) in this issue of *Structure*. This, together with a similar NMR structure by Hibbert et al. (2005) provide some insights into the function of the receptor.

The low-affinity IgE receptor, CD23, is a member of the C-type lectin receptor family that also recognizes CD21 and other ligands. While IgE binding appears to be mainly mediated by protein-protein interactions, it is less clear whether CD23 recognizes CD21 in a carbohydrate-dependent manner. In particular, does CD23 functions as a bona fide calcium-dependent lectin, like the classic mannose binding protein (MBP), selectins, and DC-SIGN, or does it merely folds like a lectin, requiring neither calcium nor carbohydrate for its function, like the natural killer cell-expressed immunoreceptors CD94 and NKG2A–D?

In general, the classic carbohydrate binding C-type lectins possess one to three conserved calcium binding sites and a sugar binding motif, EPN or QPD, specific for either mannose-type or galactose-type carbohydrates. In contrast, the known noncarbohydrate binding C-type lectin-like receptors appear to have lost all functional calcium sites as well as the sugar binding motif. By sequence, CD23 is most similar to DC-SIGN, which retains both the primary and the secondary Ca^{2+} binding sites as well as an EPN motif with preference for mannose-type sugars (Feinberg et al., 2001). At least the primary Ca^{2+} binding site appears conserved in CD23. The putative sugar binding motif appears crippled in human, but not animal, CD23 (Wurzburg et al., 2006). Functionally, both carbohydrate-dependent and -independent binding have been prescribed for this receptor. So, if it smells like a lectin and dresses like a lectin, must it a lectin ?

In this issue of *Structure*, Wurzburg et al. (2006) describe the crystal structures of the lectin domain of human CD23 in both the apo (calcium null) and the calcium bound forms, which may shed light on the function of this receptor (Figure 1). A similar structure was also determined in solution by nuclear magnetic resonance (NMR) (Hibbert et al., 2005). In the crystal structure, the authors found that only the primary Ca^{2+} binding site is occupied in human CD23 and that both Loop L1, the putative secondary calcium binding loop in MBP, and L4, the primary calcium and carbohydrate binding loop in MBP, displayed large conformational changes in response to calcium binding (see Figure 2 of Wurzburg et al. [2006]). They then proposed that this conformational change is necessary for CD23 to bind IgE, since many of the loop